International Journal of Systematic Bacteriology (1998), 48, 1031–1035 Printed in Great Britain

NOTE

Bacillus pseudomycoides sp. nov.

L. K. Nakamura

Tel: +1 309 681 6395. Fax: +1 309 681 6672. e-mail: lnakamura@sunca.ncaur.usda.gov

Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, IL 61604, USA

Previous DNA relatedness studies showed that strains identified as *Bacillus mycoides* segregated into two genetically distinct yet phenotypically similar groups, one being *B. mycoides sensu stricto* and the other, an unclassified taxon. In the present study, the taxonomic position of this second group was assessed by measuring DNA relatedness and determining phenotypic characteristics of an increased number of *B. mycoides* strains. Also determined was the second group's 16S RNA gene sequence. The 36 *B. mycoides* strains studied segregated into two genetically distinct groups showing DNA relatedness of about 30%; 18 strains represented the species proper and 18 the second group with intragroup DNA relatedness for both groups ranging from 70 to 100%. DNA relatedness to the type strains of presently recognized species with G+C contents of approximately 35 mol% (*Bacillus alcalophilus, Bacillus cereus, Bacillus circulans, Bacillus lentus, Bacillus megaterium* and *Bacillus sphaericus*) ranged from 22 to 37%. Although shown to be genetically distinct taxa, the two *B. mycoides* groups exhibited highly similar (98%) 16S RNA sequences. Phylogenetic analyses showed that both *B. mycoides* and the second group clustered closely with *B. cereus.* Although not distinguishable by physiological and morphological characteristics, the two *B. mycoides* groups and *B. cereus* were clearly separable based on fatty acid composition. The data established that the second *B. mycoides* group merits recognition as a new species for which the name *Bacillus pseudomycoides* is proposed. The type strain is NRRL B-617^T.

Keywords: *Bacillus pseudomycoides* sp. nov., 16S rDNA sequence analysis

In 1886, Flugge validly described *Bacillus mycoides* (2). This species is an aerobic, spore forming, rod-shaped, non-motile organism with a penchant to form rhizoidal colonies. Later studies showed that *Bacillus cereus* and *B. mycoides* have virtually identical physiological and biochemical characteristics (4, 9). Because of such studies, most bacteriologists regarded *B. mycoides* as a variant of *Bacillus cereus* (3, 4). Limited DNA reassociation studies suggested that *B. mycoides* may be genetically closely related to *B. cereus* (8, 10). Recent DNA relatedness analyses of a large number of *B. mycoides* and *B. cereus* strains established that these two species were genetically distinct taxa (5). Moreover, the same studies revealed two taxa within the species *B. mycoides*, one being the species *sensu stricto* and the other (group 2) a yet unnamed species. Furthermore, these two *B. mycoides* sub-groups were distinguishable by significant differences in 12:0 iso and 13:0 anteiso acids of their whole-cell fatty acid profiles (5). To establish the genetic distinctiveness of the group 2 organisms, the present study measured DNA relatedness among an increased number of *B. mycoides* strains and also assessed the relatedness of group 2 to selected *Bacillaceae*. Furthermore, the 16S rDNA of a representative strain was sequenced to determine the phylogenetic relationship of the group 2 organisms to *B. mycoides sensu stricto*, *B. cereus* and other *Bacillaceae*.

DNA relatedness of group 2 to each other, to other *B. mycoides*, and to *Bacillus* species with G+C contents of approximately 35 mol% was determined spectrophotometrically using previously described protocols (7) and the method of De Ley *et al.* (1). To the previously studied list (5) of *B. mycoides sensu lato* strains were added NRRL B-14828, NRRL B-14946, NRRL B-14947, NRRL B-14948 and NRRL B-14949. These five strains were phenotypically characterized by the method of Gordon *et al.* (3).

The GenBank accession number for the 16S rDNA sequence of strain NRRL B-617^T is AF013121.

Table 1. DNA relatedness of *Bacillus cereus* and organisms identified as *Bacillus mycoides*

Percentage reassociation values are means of two determinations; the maximum difference noted between determinations was 7%.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1. NRS-273^{T}	1·00																																				
2. B-346	0·20	1·00																																			
3. B-347	0·97	0·31	1·00																																		
4. B-615	0·73	0·37	0·73	1·00																																	
5. B-617^{T}	0·31	1·00	0·16	0·22	1·00																																
6. B-618	0·31	1·00	0·29	0·27	1·00	1·00																															
7. B-3436	0·71	0·30	0·70	0·74	0·30	0·22	1·00																														
8. B-3711^{T}	0·33	0·38	0·43	0·32	0·37	0·32	0·38	1·00																													
9. NRS-318	0·39	1·00	0·12	0·21	1·00	1·00	0·34	0·32	1·00																												
10. NRS-319	0·75	0·18	0·76	0·76	0·36	0·16	0·71	0·18	0·18	1·00																											
11. NRS-321	0·32	0·90	0·15	0·23	1·00	1·00	0·13	0·32	0·80	0·15	1·00																										
12. NRS-322	0·33	0·90	0·32	0·30	1·00	1·00	0·28	0·37	1·00	0·21	1·00	1·00																									
13. NRS-323	0·35	1·00	0·33	0·31	1·00	1·00	0·27	0·22	0·90	0·23	0·86	0·87	1·00																								
14. NRS-371	0·35	0·99	0·34	0·24	1·00	0·90	0·34	0·30	0·87	0·37	0·88	0·95	0·99	1·00																							
15. NRS-324	0·20	0·96	0·37	0·28	1·00	0·99	0·30	0·16	1·00	0·22	0·92	0·99	0·95	0·97	1·00																						
16. NRS-325	1·00	0·21	0·92	0·95	0·24	0·30	0·71	0·49	0·31	0·72	0·26	0·20	0·24	0·30	0·28	1·00																					
17. NRS-327	0·18	0·94	0·25	0·23	0·89	0·92	0·36	0·28	0·94	0·30	0·92	0·94	1·00	0·90	0·99	0·25	1·00																				
18. NRS-1316	0·96	0·27	0·98	0·99	0·29	0·26	0·73	0·40	0·27	0·78	0·26	0·38	0·33	0·27	0·31	1·00	0·31	1·00																			
19. BD-2	0·98	0·19	0·89	0·97	0·30	0·32	0·71	0·37	0·29	0·70	0·20	0·15	0·24	0·25	0·28	0·83	0·26	0·97	1·00																		
20. BD-3	0·74	0·20	0·74	0·70	0·31	0·30	0·73	0·33	0·25	0·74	0·21	0·29	0·31	0·28	0·27	0·70	0·19	0·76	0·71	1·00																	
21. BD-5	0·35	0·72	0·27	0·22	0·71	0·79	0·32	0·27	0·73	0·27	0·78	0·70	0·78	0·70	0·70	0·22	0·71	0·30	0·20	0·22	1·00																
22. NRS-306	0·73	0·24	0·74	0·76	0·28	0·27	0·75	0·28	0·20	0·72	0·26	0·33	0·31	0·30	0·27	0·72	0·28	0·70	0·73	0·70	0·18	1·00															
23. BD-4	0·80	0·25	0·82	0·93	0·30	0·28	0·71	0·32	0·27	0·72	0·30	0·29	0·30	0·30	0·33	0·90	0·30	0·85	0·89	0·72	0·20	0·71	1·00														
24. BD-6	0·34	0·72	0·34	0·18	0·71	0·72	0·20	0·36	0·70	0·25	0·76	0·73	0·76	0·71	0·79	0·24	0·74	0·30	0·30	0·25	0·97	0·27	0·30	1·00													
25. BD-7	0·83	0·29	1·00	0·98	0·22	0·30	0·72	0·30	0·31	0·70	0·27	0·38	0·35	0·28	0·25	0·82	0·33	0·89	0·82	0·71	0·25	0·75	0·88	0·31	1·00												
26. BD-9	0·76	0·22	0·70	0·73	0·30	0·36	0·75	0·34	0·27	0·71	0·24	0·11	0·32	0·33	0·19	0·60	0·39	0·76	0·77	0·72	0·27	0·95	0·76	0·30	0·71	1·00											
27. BD-14	0·20	0·74	0·36	0·27	0·72	0·78	0·24	0·40	0·79	0·27	0·75	0·72	0·77	0·73	0·71	0·30	0·72	0·31	0·29	0·28	1·00	0·23	0·29	1·00	0·29	0·29	1·00										
28. BD-15	0·72	0·27	0·71	0·79	0·29	0·32	0·75	0·44	0·32	0·70	0·21	0·43	0·38	0·31	0·30	0·90	0·26	0·76	0·70	0·73	0·30	0·96	0·71	0·34	0·70	0·92	0·27	1·00									
29. BD-10	0·45	0·70	0·30	0·29	0·74	0·77	0·27	0·41	0·71	0·33	0·75	0·62	0·74	0·78	0·79	0·22	0·73	0·32	0·29	0·23	0·99	0·30	0·24	1·00	0·26	0·29	1·00	0·24	1·00								
30. BD-12	0·75	0·34	0·71	0·74	0·23	0·28	0·70	0·39	0·33	0·71	0·27	0·30	0·39	0·37	0·35	0·72	0·30	0·79	0·73	0·70	0·29	1·00	0·71	0·27	0·70	1·00	0·35	0·79	0·30	1·00							
31. BD-18	0·72	0·26	0·74	0·71	0·21	0·25	0·75	0·37	0·30	0·72	0·23	0·27	0·35	0·29	0·30	0·76	0·35	0·67	0·70	0·76	0·30	0·99	0·77	0·30	0·72	1·00	0·30	0·93	0·26	0·94	1·00						
32. BD-23	0·97	0·29	1·00	0·91	0·30	0·34	0·73	0·35	0·28	0·77	0·21	0·29	0·30	0·31	0·37	1·00	0·29	0·94	0·88	0·72	0·36	0·77	1·00	0·25	0·97	0·70	0·31	0·70	0·28	0·76	0·77	1·00					
33. B-14946	0·39	0·85	0·29	0·30	1·00	1·00	0·27	0·33	1·00	0·31	0·99	0·96	0·91	1·00	0·94	0·36	0·99	0·32	0·27	0·34	0·80	0·28	0·31	0·79	0·27	0·29	0·76	0·31	0·70	0·23	0·31	0·33	1·00				
34. B-14947	0·21	0·93	0·25	0·25	0·97	0·95	0·23	0·36	0·97	0·30	0·99	0·95	1·00	0·99	0·96	0·31	1·00	0·32	0·26	0·35	0·82	0·30	0·37	0·77	0·28	0·25	0·78	0·27	0·76	0·29	0·26	0·29	1·00	1·00			
35. B-14948	0·38	0·90	0·34	0·32	0·99	0·93	0·30	0·31	0·91	0·33	1·00	0·97	0·93	0·98	1·00	0·29	0·93	0·31	0·31	0·31	0·77	0·33	0·33	0·78	0·30	0·32	0·73	0·26	0·73	0·30	0·24	0·28	1·00	0·99	1·00		
36. B-14949	0·27	0·99	0·30	0·36	1·00	0·99	0·33	0·29	0·96	0·37	1·00	0·91	1·00	1·00	0·93	0·30	0·97	0·27	0·30	0·25	0·78	0·35	0·32	0·73	0·36	0·34	0·77	0·30	0·71	0·31	0·27	0·34	0·99	0·97	0·98	1·00	
37. B-14828	1·00	0·33	0·94	0·81	0·32	0·29	0·74	0·72	0·34	0·71	0·34	0·27	0·30	0·37	0·33	0·98	0·32	0·89	0·90	0·74	0·31	0·70	0·96	0·34	0·93	0·77	0·36	0·72	0·27	0·71	0·73	0·90	0·31	0·28	0·32	0·33	1·00

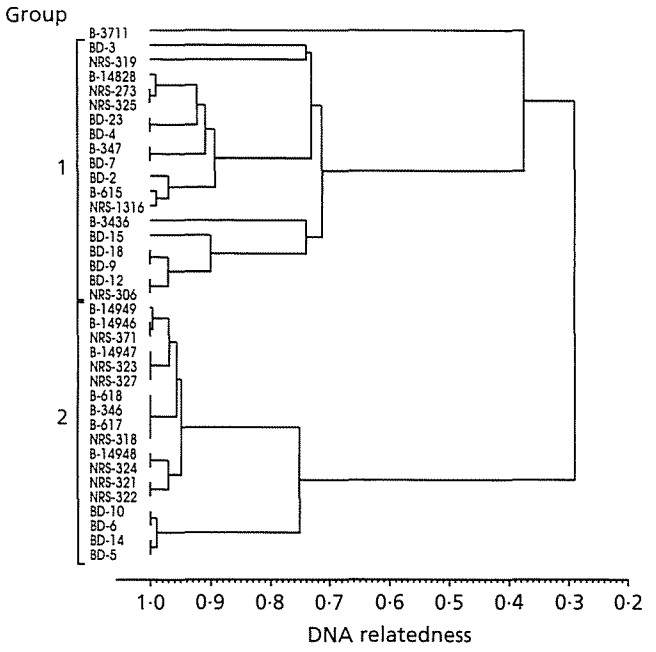

Fig. 1. Dendrogram showing the levels of DNA relatedness among *B. mycoides* strains based on unweighted pair group method with arithmetic average-linkage clustering algorithm (11). All strain designations are NRRL numbers. Clustering computations were carried out using the PC-SAS version 6.04 (SAS Institute, Cary, NC, USA) SAS/STAT cluster procedure. The dendrogram was generated with SAS/GRAPH, using the SAS macro GRAFTREE written and provided by Dan Jacobs, University of Maryland.

The phylogenetic relationship of the two *B. mycoides* sub-groups was resolved from 16S rRNA gene sequence analysis of NRRL B-617^T, a representative group 2 strain. The procedures for PCR amplification and DNA sequencing were described previously (6). Resulting sequence data were subjected, together with existing *Bacillaceae* sequences obtained from GenBank, to similarity and phylogenetic analyses with protocols and computer programs described earlier (6). Bacterial strains compared in this study and their 16S rDNA sequence GenBank accession numbers (in parentheses) are: *Alicyclobacillus cycloheptanicus* ATCC 49028^T (X51928), *Bacillus cereus* IAM 12605^T (D16266), *Bacillus circulans* IAM 12462^T (D78312), *Bacillus coagulans* JCM 2257^T (D78313), *Bacillus globisporus* NCIMB 11434^T, *Bacillus licheniformis* DSM 13^T (X68416), *Bacillus megaterium* IAM 13418^T (D16273), *B. mycoides* DSM 2048^T (X55061), *Bacillus pasteurii* NCIMB 8841^T (X60631), *Bacillus sphaericus* IAM 13420^T (D16280), *Bacillus subtilis* NCDO 1769^T (X60646), *Bacillus stearothermophilus* NRS-T10 (57309), *Bacillus thermoglucosidasius* ATCC 43742^T (X60641), *Brevibacillus brevis* JCM 2503^T (D78457), *Brevibacillus laterosporus* JDM 2496^T (D78461), *Paenibacillus azotofixans* NRRL B-14372^T (D78318), *Paenibacillus larvae* subsp. *larvae* ATCC 9545^T (X60619), *Paenibacillus polymyxa* IAM 13419 (D16276) and *Escherichia coli* ATCC 11775^T (X80725).

Data in Table 1 show the extent of DNA reassociation measured among strains identified as *B. mycoides*. Fig. 1 is a dendrogram constructed from the data in Table 1. This dendrogram shows that *B. mycoides sensu lato* segregated into two groups related approximately at the 30% level. Such low relatedness indicated that *B. mycoides sensu stricto* (group 1) and group 2 were genetically distinct taxa. [Introduction of five additional strains caused minor linkage and compositional changes in groups shown in a comparable dendrogram presented earlier (5). Because NRRL NRS-1216 had no significance in this study, it was removed.] Moreover, DNA relatedness showed that group 2 was not closely related genetically to other *Bacillus* species with similar G+C contents (35 mol%), namely, *B. alcalophilus*, *B. circulans*, *B. lentus*, *B. megaterium* and *B. sphaericus* (Table 2).

Table 2. DNA relatedness of group 2 organisms to selected *Bacillus* species

Values are means of two determinations; the maximum difference observed between deteminations was 6%.

Strains	Reassociation (%) with DNA from group 2 strains			
	B-346	B-617^T	NRS-318	B-14949
B. alcalophilus NRS-1548^T	25	24	26	29
B. cereus B-3711^T	33	37	32	30
B. circulans B-380^T	31	33	31	33
B. lentus B-396^T	30	26	27	28
B. megaterium B-14308^T	22	24	30	27
B. sphaericus NRS-348	23	28	30	30
B. mycoides NRS-273^T	30	31	35	27

Table 3. Phenotypic comparison of the unknown taxon and selected *Bacillus* species with G+C contents of about 35 mol%

Data for selected species were taken from reference 3; five strains were characterized in the present study. +, Positive reaction; −, negative reaction; v, variable reaction.

Characteristic	Unknown*	*B. alcalophilus*	*B. circulans*	*B. lentus*	*B. megaterium*	*B. sphaericus*
Spore shape	Ellipse	Ellipse	Ellipse	Ellipse	Ellipse	Round
Swollen sporangium	−	+	+	−	−	+
Motility	−	+	+	+	+	+
Anaerobic growth	+	−	+	−	−	−
Acetoin produced	+	−	−	−	−	−
Acid from:						
D-Glucose	+	+	+	+	+	−
L-Arabinose	−	+	+	+	v	−
D-Xylose	−	+	+	+	v	−
D-Mannitol	−	+	+	+	v	−
Casein hydrolysis	+	+	+	v	+	v
Starch hydrolysis	+	+	+	+	+	−
Citrate utilization	+	−	v	−	+	v
Tyrosine degradation	+	−	−	−	v	−
Lecithinase	+	−	−	−	−	−
$NO_3^- \rightarrow NO_2^-$	+	−	v	v	v	−
Growth at pH 5·7	+	−	v	−	v	v
Growth in 7% NaCl	−	−	v	v	v	−

*Unknown (group 2), *B. mycoides* and *B. cereus* have identical characteristics.

A collation of data from the present and previous studies (Table 3) also demonstrated that the unnamed species was phenotypically distinct from *Bacillus* species with G+C contents of 35 mol%. The key differentiating characteristics of the unknown were positive responses for acetylmethylcarbinol production, lecithinase activity and anaerobic growth, and a negative response for motility. All the other species were motile and negative for the first two characteristics; only *B. circulans* was facultatively anaerobic. Carbohydrate fermentation pattern, citrate utilization, and tyrosine degradation were also useful characteristics for the differentiation process.

The phylogenetic relationship of group 2 to other *Bacillaceae* was determined from 16S sequence information. Sequence similarity calculations showed high correspondence (98·2–99·4%) of 16S rDNA sequences of *B. cereus* and the *B. mycoides* sub-groups showing close phylogenetic relatedness of these taxa. This observation was corroborated in the phylogenetic tree (Fig. 2) where the three taxa formed a close cluster within a clade consisting of other *Bacillus* species. Group 2 (represented by NRRL B-617^T) appeared as a distinct taxon that was more distantly related to *B. cereus* than to *B. mycoides sensu stricto*.

Based on the results of previous (5) and present studies, group 2 unknowns merit recognition as members of a new species for which the name *Bacillus pseudomycoides* is proposed. A description of the species is given below.

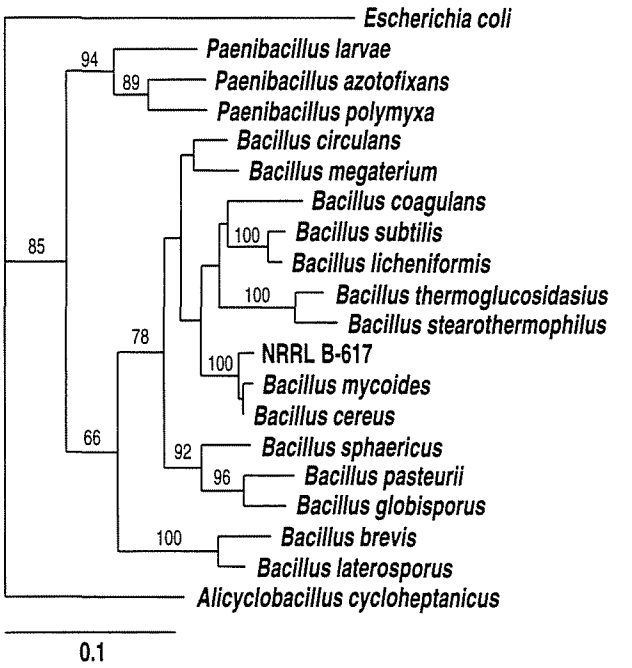

Fig. 2. Tree showing the phylogenetic position of NRRL B-617^T among representative *Bacillaceae*. The tree, which was rooted by using *E. coli* as the outgroup, was generated by maximum-likelihood (DNAml). Trees generated by parsimony analysis (DNAPARS) had similar topologies. Bootstrap values are given for each node having 60% or greater support; the values (100 data sets) were determined by SEQBOOT program. DNAML, DNAPARS and SEQBOOT were part of the PHYLIP, version 3.55c, software package (J. Felsenstein, University of Washington, Seattle, USA). Bar, 0·1 accumulated changes per nucleotide.

Bacillus pseudomycoides sp. nov.

Bacillus pseudomycoides (pseu.do.my.co.i'des. Gr. adj. *pseudes* false; M.L. adj. *mycoides* fungus-like; M.L. adj. *pseudomycoides* false fungus-like).

Vegetative cells are non-motile 1·0 μm wide and 3·0–5·0 μm long (as determined by phase microscopy) and occur singly and in short chains. They produce ellipsoidal spores in sporangia that are not distended. Gram-positive. Agar colonies are white to cream, opaque, and usually rhizoidal. Catalase is produced. Oxidase is not produced. Facultatively anaerobic. Acetylmethylcarbinol (Voges–Proskauer test) is produced. pH values in Voges–Proskauer test range from 4·5 to 5·6. Indole, dihydroxyacetone and hydrogen sulfide are not formed. Nitrate is reduced to nitrite. Citrate utilization is variable; propionate is not utilized. Starch, casein, tyrosine and egg-yolk lecithin are decomposed. Litmus milk is alkaline, reduced and decomposed. Growth occurs in 0·001 % lysozyme, 7 % NaCl and at pH 5·7. The optimum growth temperature is 28 °C, the maximum, 40 °C, and the minimum, 15 °C. Acid but no gas is produced from D-glucose; L-arabinose, D-mannitol, and D-xylose are not fermented. Distinguished from *Bacillus mycoides* by differences in 12:0 iso and 13:0 anteiso fatty acid levels; distinguished from *Bacillus cereus* by differences in 12:0 iso, 12:0, 15:0 iso and 16:0 fatty acid composition. G+C content ranges from 34 to 36 mol%. Isolated mainly from soil. The type strain, NRRL B-617^T, is deposited in the Agricultural Research Service Culture Collection, Peoria, IL, USA.

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